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Markareetta E. Törnwall, Jarmo Virtamo, Pasi A. Korhonen, Mikko J. Virtanen, Demetrius Albanes and Jussi K. Huttunen

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Postintervention Effect of Alpha Tocopherol and Beta Carotene on Different Strokes

A 6-Year Follow-Up of the Alpha Tocopherol, Beta Carotene Cancer Prevention Study

Markareetta E. Törnwall, MD, PhD; Jarmo Virtamo, MD, PhD; Pasi A. Korhonen, PhD; Mikko J. Virtanen, MSc; Demetrius Albanes, MD; Jussi K. Huttunen, MD, PhD

Background and Purpose—In the Alpha Tocopherol, Beta Carotene Cancer Prevention Study, alpha tocopherol supplementation decreased risk of cerebral infarction by 14% (95% CI, –25% to –1%), and beta carotene increased risk of intracerebral hemorrhage by 62% (95% CI, 10% to 132%). We report here the 6-year postintervention effects of alpha tocopherol and beta carotene supplementation on stroke and its subtypes.

Methods—A total of 29 133 male smokers, aged 50 to 69 years, were randomized to receive 50 mg of alpha tocopherol, 20 mg of beta carotene, both, or placebo daily for 5 to 8 years. At the beginning of the post-trial follow-up, 24 382 men were still at risk for first-ever stroke. During the post-trial follow-up, 1327 men experienced a stroke: 1087 cerebral infarctions, 148 intracerebral hemorrhages, 64 subarachnoid hemorrhages, and 28 unspecified strokes.

Results—Post-trial risk for cerebral infarction was elevated among those who had received alpha tocopherol compared with those who had not (relative risk [RR], 1.13; 95% CI, 1.00 to 1.27), whereas beta carotene had no effect (RR, 0.97; 95% CI, 0.86 to 1.09). Alpha tocopherol supplementation was associated with a postintervention RR of 1.01 (95% CI, 0.73 to 1.39) for intracerebral hemorrhage and 1.38 (95% CI, 0.84 to 2.26) for subarachnoid hemorrhage. The corresponding RRs associated with beta carotene supplementation were 1.38 (95% CI, 0.99 to 1.91) and 1.09 (95% CI, 0.67 to 1.77), respectively.

Conclusions—Neither alpha tocopherol nor beta carotene supplementation had any postintervention preventive effects on stroke. The post-trial increase in cerebral infarction risk among recipients of alpha tocopherol may present a rebound of the reduced risk of cerebral infarction during the intervention. (*Stroke*. 2004;35:1908-1913.)

Key Words: stroke ■ primary prevention ■ randomized controlled trials ■ antioxidants

Stroke is a leading cause of death in the developed countries. It is also a major cause of long-term disability, and the economic burden of it will increase in the future.¹ Any possibilities of preventing stroke should be explored thoroughly. The possible preventive effects of antioxidant vitamins on chronic diseases have been under investigation for several decades. Large controlled trials have tested the effect of antioxidant supplementation on cardiovascular diseases. However, in contrast to expectations on the basis of observational studies, antioxidant vitamins have not been effective in prevention of cardiovascular diseases.²

Alpha tocopherol, the main constituent of vitamin E, is a potent chain-breaking lipid-soluble antioxidant.³ Beyond its antioxidant activity, it reduces platelet adhesion and aggregation.^{4,5} Furthermore, its metabolite inhibits vitamin K–dependent carboxylase, acting as an anticoagulant.⁶ The clinical importance of these antiplatelet and anticlotting properties is

obscure because no effect has been observed in some studies.⁷ Beta carotene, also a lipid-soluble antioxidant, acts as a singlet oxygen scavenger and may regenerate the alpha-tocopheroxyl radical into alpha tocopherol.⁸

In the Alpha Tocopherol, Beta Carotene Cancer Prevention (ATBC) Study, alpha tocopherol supplementation decreased the risk of cerebral infarction by 14%, and beta carotene supplementation increased the risk of intracerebral hemorrhage by 62%.⁹ We report here the 6-year postintervention findings of alpha tocopherol and beta carotene supplementation on risk for stroke and its subtypes.

Subjects and Methods

ATBC Study

The ATBC Study was a randomized, double-blind, placebo-controlled trial. The primary aim was to test the effect of alpha tocopherol and beta carotene supplementation on the incidence of

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From the National Public Health Institute (M.E.T., J.V., P.A.K., M.J.V., J.K.H.), Helsinki, Finland; and the National Cancer Institute (D.A.), Bethesda, Md.

Correspondence to Dr Markareetta E. Törnwall, National Public Health Institute, Department of Epidemiology and Health Promotion, Mannerheimintie 166, 00300 Helsinki, Finland. E-mail markareetta.tornwall@ktl.fi

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lung cancer, and the secondary aim was to evaluate their effects on other cancers, all-cause mortality, and cardiovascular diseases. The trial cohort was screened by using a postal survey from among a total population of 50- to 69-year-old men living in southwestern Finland ($n=290\,406$). Men who were current smokers and willing to participate in the study ($n=42\,957$) were invited to undergo baseline examinations. Exclusion criteria were previous cancer, severe angina on exertion, any other serious disease limiting long-term participation, or use of anticoagulants or vitamin E (>20 mg per day), or beta carotene (>6 mg per day) or vitamin A supplements ($>20\,000$ international units [IU] per day).¹⁰ A total of 29 133 men were randomized into 1 of 4 intervention regimens from 1985 to 1988. They received 50 mg of dl-alpha-tocopheryl acetate or 20 mg of beta carotene, or both, or placebo in 1 daily capsule until April 1993. The ATBC Study was approved by the institutional review boards of the National Public Health Institute in Helsinki, Finland, and the National Cancer Institute in Bethesda, Md.

Before randomization, medical background information was collected, blood pressure was measured, and a blood sample was drawn. Men were informed about the trial and they signed the consent form. During the intervention, participants had a follow-up visit 3 times per year. At every visit, men returned the pack with the remaining study capsules and received a new supply. Overall capsule compliance was estimated by dividing the number of capsules taken by the number of days in the trial. Median capsule compliance was 99% among active participants in all supplementation groups. The design and methods of the ATBC Study have been described in detail previously.¹⁰

Subjects

At the beginning of the post-trial follow-up, 24 382 participants were still at risk for first-ever stroke. They were followed up for first-ever stroke through April 30, 1999 (6-year post-trial follow-up).

End Points

Cases were identified from the National Hospital Discharge Register and from the Register of Causes of Death, which cover $>90\%$ of acute stroke events in Finland.¹¹ Both registers use the codes of the International Classification of Diseases (ICD). Stroke was defined as ICD-9 codes (used until 1996) 430, 431, 433, 434, 436 (4330X, 4331X, 4339X, and 4349X excluded) and ICD-10 codes I60, I61, I63, I64. For cerebral infarction, we searched for ICD-9 codes 433 to 434 and ICD-10 code I63. For intracerebral hemorrhage, we searched for ICD-9 code 431 and ICD-10 code I61 and for subarachnoid hemorrhage, ICD-9 code 430 and ICD-10 code I60. Stroke was considered fatal if any type of stroke led to death within 90 days from onset of attack. A validation study on the stroke diagnoses of the registers was done for the trial stroke cases using standard diagnostic criteria.¹² The diagnoses were valid in 90% of all strokes (including unspecified strokes), in 90% of cerebral infarctions, and in 79% of subarachnoid and 82% of intracerebral hemorrhages.

Statistical Analysis

Censoring was defined as either death or end of follow-up (April 30, 1999) and assumed to be independent of the end point. Crude rates per 1000 person years were calculated for incidence of stroke, cerebral infarction, intracerebral hemorrhage, and subarachnoid hemorrhage in each of the 4 intervention groups and according to the 2×2 factorial design. Relative risk (RR) point estimates and their 95% CIs were obtained using a Poisson regression model.¹³ Interaction between post-trial effects of alpha tocopherol and beta carotene for stroke and its subtypes was tested by comparing the main effects only and 4 group interaction models using likelihood ratio tests. No significant interactions were observed. Number of fatal events was calculated for each stroke type, and difference in fatality between the trial supplementation groups was estimated by χ^2 test.

To estimate the calendar time-specific RRs, we calculated smoothed RR estimates and their 95% pointwise CIs using a generalized additive model.¹⁴ We first divided calendar time into monthly intervals with the exception that we combined calendar time

until April 1986 for the first interval because the risk sets were small at this earliest phase of the recruitment period that started in 1985. The monthly rates were treated as Poisson responses. For each target time point, 40% of all monthly observations nearest to the target were used to define a neighborhood for which a weighted linear curve was used to estimate the RR at the target point. The weights for the monthly observations around the target point were calculated from a tricube kernel centered at the target point.

Results

At the beginning of the post-trial follow-up, the average age of the 24 382 men at risk for first-ever stroke was 63.5 years. At the baseline of the trial between 1985 and 1988, they smoked an average of 20 cigarettes daily, had smoked for 36 years, their systolic blood pressure was 140 mm Hg and diastolic 88 mm Hg, total cholesterol was 6.17 mmol/L, and HDL-cholesterol 1.12 mmol/L. The baseline characteristics of the 24 382 men were similar across the 4 supplementation groups

Total Stroke

During the 6-year post-trial follow-up, 1327 first-ever strokes occurred. The rate per 1000 person years in the 4 study groups varied between 9.42 and 11.17 (Table 1). The post-trial risk for stroke was significantly higher among those who had received alpha tocopherol compared with those who had not (RR, 1.12; 95% CI, 1.01 to 1.25). Beta carotene had no post-trial effect on risk for stroke (Table 2). Of the 1327 strokes, 305 (23%) were fatal within 90 days. The fatality did not differ among those who had received alpha tocopherol (22% versus 25%; $P=0.23$) or beta carotene (25% versus 21%; $P=0.08$) compared with those who had not.

Cerebral Infarction

Four of 5 strokes were cerebral infarctions ($n=1087$). The rate per 1000 person years varied between 7.62 and 8.91 in the 4 study groups (Table 1). The post-trial risk of cerebral infarction was elevated significantly among those who had received alpha tocopherol compared with those who had not (RR, 1.13; 95% CI, 1.00 to 1.27), whereas beta carotene had no post-trial effect (Table 2). The smoothed calendar time-specific RRs of cerebral infarction were <1.0 during the intervention but increased to >1.0 2 years after stopping the supplementation among those who had been alpha tocopherol recipients compared with nonrecipients (Figure 1). At the end of the post-trial follow-up, the cumulative frequencies since the start of supplementation were equal (6.6% among the recipients and the nonrecipients of alpha tocopherol; data not shown). Among the beta carotene recipients compared with nonrecipients, the RRs were near 1.0 except during the last 3 post-trial years, when the smoothed RRs fell to <1.0 (Figure 1). The 90-day fatality of cerebral infarction ($n=183$) was smaller among those who had received alpha tocopherol compared with those who had not (14% versus 20%; $P=0.03$). The corresponding numbers for beta carotene were 19% versus 15% ($P=0.09$).

Hemorrhagic Stroke

Hemorrhagic stroke events consisted of 148 intracerebral hemorrhages and 64 subarachnoid hemorrhages. Rate per 1000 person years of intracerebral hemorrhage varied be-

TABLE 1. Incidence and RR of Total Stroke and Its Subtypes During 6-Year Post-Trial Follow-Up by Regimen

	Placebo	Alpha Tocopherol	Alpha Tocopherol and Beta Carotene	Beta Carotene
No. at risk	6154	6114	6061	6053
All stroke				
No. of incident events	325	340	359	303
Rate per 1000 person years	9.85	10.41	11.17	9.42
RR (95% CI)	1.00	1.06 (0.91–1.23)	1.13 (0.98–1.32)	0.96 (0.82–1.12)
Cerebral infarction				
No. of incident events	267	291	284	245
Rate per 1000 person years	8.09	8.91	8.83	7.62
RR (95% CI)	1.00	1.10 (0.93–1.30)	1.09 (0.92–1.29)	0.94 (0.79–1.12)
Intracerebral hemorrhage				
No. of incident events	36	27	47	38
Rate per 1000 person years	1.09	0.83	1.46	1.18
RR (95% CI)	1.00	0.76 (0.46–1.25)	1.34 (0.87–2.07)	1.08 (0.69–1.71)
Subarachnoid hemorrhage				
No. of incident events	13	18	19	14
Rate per 1000 person years	0.39	0.55	0.59	0.44
RR (95% CI)	1.00	1.40 (0.69–2.85)	1.50 (0.74–3.04)	1.10 (0.52–2.35)

tween 0.83 and 1.46, and that of subarachnoid hemorrhage varied between 0.30 and 0.59 in the 4 study groups (Table 1). Alpha tocopherol supplementation had no effect on post-trial risk for intracerebral hemorrhage, whereas there was a suggestion of elevated risk among those who had received beta carotene compared with those who had not (RR, 1.38; 95% CI, 0.99 to 1.91; Table 2). The smoothed RRs of the alpha tocopherol recipients compared with nonrecipients fluctuated at ≈ 1.0 , whereas the RRs were >1.0 for recipients of beta

carotene compared with nonrecipients during intervention and post-trial period (Figure 2). Of the intracerebral hemorrhages, 84 were fatal. The fatality rate did not differ among those who had been alpha tocopherol recipients compared with nonrecipients (59% versus 54%; $P=0.62$) or beta carotene recipients compared with nonrecipients (54% versus 60%; $P=0.50$).

For subarachnoid hemorrhage, post-trial risk was nonsignificantly elevated among those who had received alpha

TABLE 2. Incidence and RR of Total Stroke and Its Subtypes During 6-Year Post-Trial Follow-Up by Alpha Tocopherol or Beta Carotene Supplementation

	No Alpha Tocopherol	Alpha Tocopherol	No Beta Carotene	Beta Carotene
No. at risk	12 207	12 175	12 268	12 114
All stroke				
No. of incident events	628	699	665	662
Rate per 1000 person years	9.64	10.79	10.13	10.29
RR (95% CI)	1.00	1.12 (1.01–1.25)	1.00	1.02 (0.91–1.13)
Cerebral infarction				
No. of incident events	512	575	558	529
Rate per 1000 person years	7.86	8.87	8.50	8.22
RR (95% CI)	1.00	1.13 (1.00–1.27)	1.00	0.97 (0.86–1.09)
Intracerebral hemorrhage				
No. of incident events	74	74	63	85
Rate per 1000 person years	1.14	1.14	0.96	1.32
RR (95% CI)	1.00	1.01 (0.73–1.39)	1.00	1.38 (0.99–1.91)
Subarachnoid hemorrhage				
No. of incident events	27	37	31	33
Rate per 1000 person years	0.41	0.57	0.47	0.51
RR (95% CI)	1.00	1.38 (0.84–2.26)	1.00	1.09 (0.67–1.77)

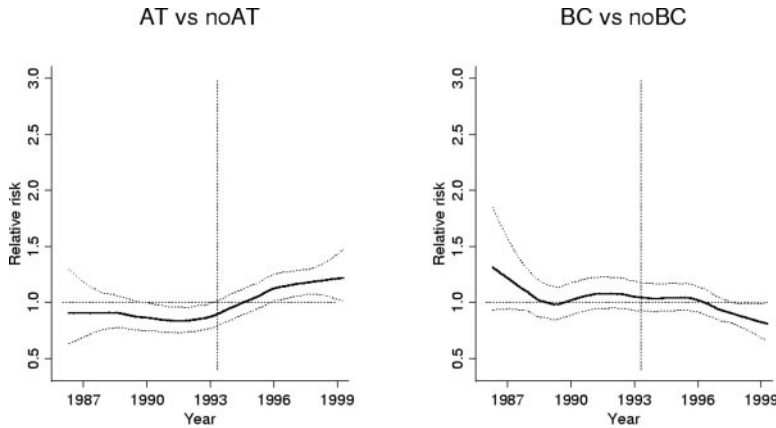


Figure 1. Smoothed RR curves of cerebral infarction by calendar time. The dotted curves refer to 95% CI. The vertical line refers to the end of trial April 30, 1993. AT indicates alpha tocopherol; noAT, no alpha tocopherol; BC, beta carotene; noBC, no beta carotene.

tocopherol compared with those who had not (1.38; 95% CI, 0.84 to 2.26). Beta carotene supplementation showed no post-trial effect (Table 2). The smoothed RRs for subarachnoid hemorrhage among alpha tocopherol recipients was >1.0 but returned to 1.0 by the end of post-trial follow-up, whereas the RRs of the beta carotene recipients compared with nonrecipients fluctuated at ≈ 1.0 (Figure 3). There were 31 fatal subarachnoid hemorrhages. The fatality rate of subarachnoid hemorrhages was higher among those who had received alpha tocopherol compared with those who had not (59% versus 33%; $P=0.047$), whereas beta carotene had no effect (48% versus 48%).

Discussion

The 6-year post-trial risk of cerebral infarction was 13% (95% CI, 0% to 27%) higher among those who had received alpha tocopherol during the trial compared with those who had not. This finding was the opposite of the result from the intervention period, during which a 14% (95% CI, -25% to -1%) reduction in risk of cerebral infarction was observed among alpha tocopherol recipients compared with nonrecipients.⁹ The lower risk of cerebral infarction among recipients of alpha tocopherol reversed in ≈ 2 years post-trial, and the favorable effect of alpha tocopherol on cerebral infarction had fully disappeared by the end of the 6-year post-trial follow-up. These temporal changes suggest that the increased post-trial risk of cerebral infarction was a rebound to the reduced risk of cerebral infarction during trial period. During the intervention period, the preventive effect of alpha tocoph-

erol emerged in ≈ 2 years,⁹ the same time it took to disappear after stopping supplementation. The preventive mechanism of alpha tocopherol is unclear, but it might be attributable to inhibition of platelet adhesion and aggregation and vascular smooth muscle cell proliferation.¹⁵ When the supplementation effect of alpha tocopherol had passed, the risk of cerebral infarction might have increased to higher than usual for several years, especially in men who had benefited from supplementation. However, because the effects of alpha tocopherol were small, they may also have appeared by chance. The trial also included only smokers; therefore, the results may not be directly generalizable to females and nonsmokers.

Risk for intracerebral hemorrhage among those who had received beta carotene diminished from 1.62 (95% CI, 1.10 to 2.36) of the intervention period⁹ to 1.38 (95% CI, 0.99 to 1.91) during the post-trial follow-up. We do not know of a mechanism to explain how beta carotene could increase the risk of intracerebral hemorrhage.

Alpha tocopherol supplementation nonsignificantly elevated the risk for subarachnoid hemorrhage during trial (RR, 1.50; 95% CI, 0.97 to 2.32)⁹ as well as post-trial (RR, 1.38; 95% CI, 0.84 to 2.26). Approximately 60% of subarachnoid hemorrhage cases among the alpha tocopherol supplemented were fatal within 90 days during trial⁹ and after trial, whereas only 30% of the cases among the nonsupplemented were fatal. Some amount of alpha tocopherol might have been stored in tissues during several years of supplementation, and the release of the stored alpha tocopherol might have main-

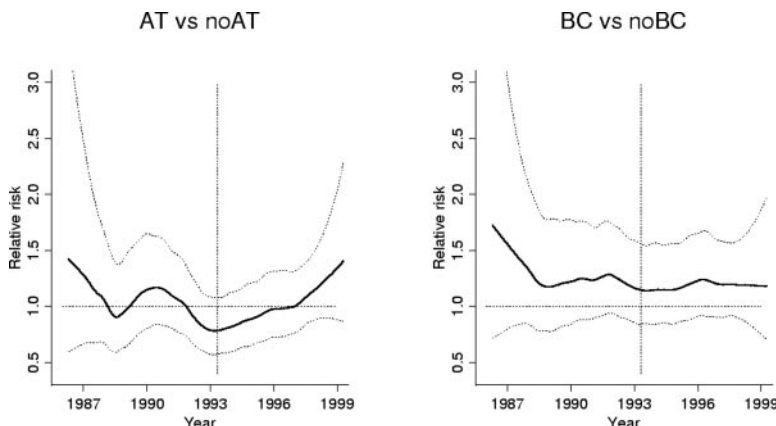


Figure 2. Smoothed RR curves of intracerebral hemorrhage by calendar time. The dotted curves refer to 95% CI. The vertical line refers to the end of trial April 30, 1993. AT indicates alpha tocopherol; noAT, no alpha tocopherol; BC, beta carotene; noBC, no beta carotene.

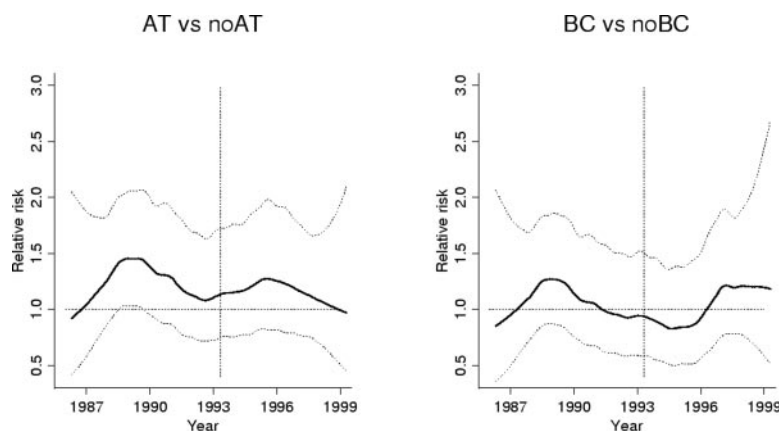


Figure 3. Smoothed RR curves of subarachnoid hemorrhage by calendar time. The dotted curves refer to 95% CI. The vertical line refers to the end of trial April 30, 1993. AT indicates alpha tocopherol; noAT, no alpha tocopherol; BC, beta carotene; noBC, no beta carotene.

tained the antiplatelet effect for some time after stopping supplementation. Whether alpha tocopherol might have some long-lasting effect on vascular wall at rupture-prone areas is unknown.

Several large clinical trials of antioxidant vitamins and cardiovascular disease have been conducted, but so far, no postintervention reports are available with which to compare our findings. Three trials with alpha tocopherol supplementation showed no significant effect on total stroke. In the double-blind Heart Outcomes Prevention Evaluation Study, subjects with cardiovascular risk factors were assigned to receive either 400 IU of alpha tocopherol or matching placebo units daily for 4.5 years. The RR of stroke was 1.17 (95% CI, 0.95 to 1.42) among alpha tocopherol recipients compared with nonrecipients. There were 17 events of hemorrhagic stroke in the alpha tocopherol group and 13 in the placebo group.¹⁶ In 2 open-labeled trials (ie, the GISSI-Prevenzione Trial and the Primary Prevention Project [PPP]) 300 mg of alpha tocopherol was administered daily for ≈ 3.5 years among patients with recent myocardial infarction and subjects at high risk for cardiovascular disease, respectively. RR of stroke was 0.87 (95% CI, 0.65 to 1.17) in the GISSI Trial and 1.24 (95% CI, 0.66 to 2.31) in the PPP among alpha tocopherol recipients compared with nonrecipients.^{17,18} The latest trial, the Heart Protection Study (HPS), allocated high-risk subjects to receive antioxidant vitamin supplementation (600 mg of vitamin E, 250 mg of ascorbic acid, and 20 mg of beta carotene daily) or matching placebo for 5 years. No difference was observed for total stroke between the antioxidant group and the placebo group (RR, 0.99; 95% CI, 0.87 to 1.12).¹⁹ Nor were differences observed for ischemic stroke (345 versus 354 cases) or hemorrhagic stroke (51 versus 53 cases, including 13 versus 7 subarachnoid hemorrhages). A meta-analysis of these trials reported an odds ratio of 1.02 (95% CI, 0.92 to 1.12) between subjects treated and not treated with vitamin E.²

The effect of beta carotene alone has been evaluated in 2 controlled trials. In the Physicians' Health Study (PHS), 50 mg of beta carotene on alternate days had no effect on risk for stroke (RR, 0.96; 95% CI, 0.83 to 1.11 [367 versus 382 events]) during 12 years of follow-up.²⁰ In the Women's Health Study (WHS), the beta carotene component, 50 mg on alternate days, was terminated early because of the findings of other trials that beta carotene may be associated with

harmful effects. Risk for stroke was nonsignificantly elevated (RR, 1.42; 95% CI, 0.96 to 2.10) among those who had received beta carotene for 2 years and followed up for 2 extra years after supplementation (61 versus 43 cases, respectively).²¹ In the HPS, PHS, and WHS trials combined, the rate of stroke did not differ among subjects treated and not treated with beta carotene (odds ratio, 1.00; 95% CI, 0.91 to 1.09).²

In conclusion, our finding of increased post-trial risk of cerebral infarction among those who had received alpha tocopherol supplementation does not contradict the possibility that alpha tocopherol may delay the occurrence of cerebral infarction. The decreased risk observed during intervention reversed in ≈ 2 years after ceasing the supplementation, and the favorable effect had fully disappeared by the end of the 6-year post-trial follow-up. However, other trials have not found any effect from alpha tocopherol supplementation on stroke, thus our intervention and post-trial effects of alpha tocopherol ought to be considered with caution because a chance finding cannot be ruled out. The observations of the effect of alpha tocopherol on subarachnoid hemorrhage and of beta carotene on intracerebral hemorrhage are difficult to explain because of the absence of any plausible mechanism. Therefore, more data from other intervention trials are needed to demonstrate the role of antioxidant vitamins in the pathogenesis of different strokes.

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References

- Goldstein LB, Adams R, Becker K, Furberg CD, Gorelick PB, Hademenos G, Hill M, Howard G, Howard VJ, Jacobs B, Levine SR, Mosca L, Sacco RL, Sherman DG, Wolf PA, del Zoppo GJ. Primary prevention of ischemic stroke. A statement for healthcare professionals from the Stroke Council of the American Heart Association. *Stroke*. 2001; 32:280–299.
- Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet*. 2003;361:2017–2023.
- Meydani M. Vitamin E. *Lancet*. 1995;345:170–175.
- Steiner M. Influence of vitamin E on platelet function in humans. *J Am Coll Nutr*. 1991;10:466–473.
- Freedman JE, Farhat JH, Loscalzo J, Keaney JF Jr. Alpha-tocopherol inhibits aggregation of human platelets by a protein kinase C-dependent mechanism. *Circulation*. 1996;94:2434–2440.

6. Dowd P, Zheng ZB. On the mechanism of the anticlotting action of vitamin E quinone. *Proc Natl Acad Sci U S A*. 1995;92:8171–8175.
7. Liu M, Wallmon A, Olsson-Mortlock C, Wallin R, Saldeen T. Mixed tocopherols inhibit platelet aggregation in humans: potential mechanisms. *Am J Clin Nutr*. 2003;77:700–706.
8. Böhm F, Edge R, Land EJ, McGarvey DJ, Truscott TG. Carotenoids enhance vitamin E antioxidant efficiency. *J Am Chem Soc*. 1997;119:621–622.
9. Leppälä JM, Virtamo J, Fogelholm R, Huttunen JK, Albanes D, Taylor PR, Heinonen OP. Controlled trial of alpha-tocopherol and beta-carotene supplements on stroke incidence and mortality in male smokers. *Arterioscler Thromb Vasc Biol*. 2000;20:230–235.
10. ATBC Cancer Prevention Study Group. The Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study: design, methods, participant characteristics and compliance. *Ann Epidemiol*. 1994;4:1–10.
11. Mähönen M, Salomaa V, Keskimäki I, Moltchanov V, Torppa J, Molarius A, Tuomilehto J, Sarti C. The feasibility of combining data from routine hospital discharge and causes-of-death registers for epidemiological studies on stroke. *Eur J Epidemiol*. 2000;16:815–817.
12. Leppälä JM, Virtamo J, Heinonen OP. Validation of stroke diagnosis in the National Hospital Discharge Register and the Register of Causes of Death in Finland. *Eur J Epidemiol*. 1999;15:155–160.
13. Breslow NE, Day NE. *Statistical Methods in Cancer Research*. Vol 2. Lyon, France: International Agency for Research on Cancer; 1987.
14. Hastie TJ, Tibshirani RJ. *General Additive Models. Monographs on Statistics and Applied Probability*, 43. London, UK: Chapman and Hall; 1990:136–173.
15. Azzi A, Ricciarelli R, Zingg JM. Non-antioxidant molecular functions of alpha-tocopherol (vitamin E). *FEBS Lett*. 2002;519:8–10.
16. Heart Outcomes Prevention Evaluation Study Investigators. Vitamin E supplementation and cardiovascular events in high-risk patients. *N Engl J Med*. 2000;342:154–160.
17. GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione Trial. *Lancet*. 1999;354:447–455.
18. Collaborative Group of the Primary Prevention Project. Low-dose aspirin and vitamin E in people at cardiovascular risk: a randomised trial in general practice. *Lancet*. 2001;357:89–95.
19. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20 536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002;360:23–33.
20. Hennekens CH, Buring JE, Manson JAE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridger PM, Willett W, Peto R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med*. 1996;334:1145–1149.
21. Lee IM, Cook NR, Manson JE, Buring JE, Hennekens CH. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the Women's Health Study. *J Natl Cancer Inst*. 1999;91:2102–2106.